British Journal of Pharmacology (1998) 125, 341–346
http://www.stockton-press.co.uk/bjp

P2X receptor-mediated excitation of nociceptive afferents in the normal and arthritic rat knee joint

[1]E. Dowd, [1,3]D.S. McQueen, [2]I.P. Chessell & [2]P.P.A. Humphrey

[1]Department of Pharmacology, University of Edinburgh Medical School, Edinburgh, EH8 9JZ and [2]Department of Pharmacology, Glaxo Institute of Applied Pharmacology, University of Cambridge, Cambridge, CB2 1QJ

1 We tested the hypothesis that functional P2X receptors are present on peripheral terminals of primary afferent articular nociceptors in the rat knee joint. Neural activity was recorded extracellularly from the medial articular nerve innervating the knee joint in rats anaesthetized with pentobarbitone.

2 The selective P2X receptor agonist, $\alpha\beta$ methylene ATP ($\alpha\beta$meATP), and the endogenous ligand, ATP, caused a rapid short-lasting excitation of a sub-population of C and Aδ nociceptive afferent nerves innervating normal knee joints when injected intra-arterially or intra-articularly, and this effect was antagonized by the non-selective P2 receptor antagonist PPADS.

3 Induction of a chronic (14–21 days) unilateral inflammatory arthritis of the knee joint using locally injected Freund's adjuvant neither increased or decreased responsiveness of joint nociceptors to $\alpha\beta$meATP or ATP.

4 Our results support the hypothesis that $\alpha\beta$meATP-sensitive P2X receptors are expressed on peripheral nociceptive afferents in the rat knee joint suggesting that they may be involved in the initiation of nociception and pain.

Keywords: ATP; P2X receptors; pain; nociception; sensory neurones; arthritis

Introduction

Adenosine 5′-triphosphate (ATP) causes pain when applied to a blister base in humans (Bleehan & Keele, 1977) and the nucleotide also depolarises rat dorsal root ganglion neurones *in vitro* (Jahr & Jessell, 1983), suggesting that it may play a role in nociception. The effects of extracellular ATP are mediated through ionotropic (P2X) and metabotropic (P2Y) receptors (Fredholm *et al*., 1994), and seven of each have been cloned to date (for review see North & Barnard, 1997). Indirect evidence is accumulating which supports a role for ATP in the initiation of pain by acting on putative P2X receptors expressed on nociceptive afferent nerve terminals (see Burnstock & Wood, 1996).

It has been established that mRNA for six of the seven known subtypes of P2X receptor are expressed in sensory ganglia (Collo *et al*., 1996) and the $P2X_3$ subtype is selectively expressed in these ganglia (Chen *et al*., 1995; Lewis *et al*., 1995). $P2X_3$ mRNA in rat dorsal root ganglia is localized to small diameter afferent neurones (Chen *et al*., 1995) which are commonly associated with nociception. In rat trigeminal ganglia, cell bodies of nociceptors innervating the tooth pulp are immunoreactive to antibodies for this receptor, whereas non-nociceptive neurones are not (Cook *et al*., 1997). However, the results from behavioural studies that have been performed to investigate the role of P2X receptors in nociception are conflicting. For example, the stable $P2X_1$ and $P2X_3$ receptor agonist, $\alpha\beta$ methylene ATP ($\alpha\beta$meATP), evokes nociceptive responses when injected into the rat food pad (Bland-Ward & Humphrey, 1997), but not when it is instilled into the rat eye (Dowd *et al*., 1997a).

Functional P2X receptors are thought to be expressed on some sensory afferent nerves in the rat because ATP and $\alpha\beta$meATP excite vagal and chemosensory primary afferents in this species (Trezise *et al*., 1993; Khakh *et al*., 1995; McQueen *et al*., 1997, 1998). Although there is indirect evidence that ATP excites peripheral nociceptors (Bland-Ward & Humphrey, 1997), there is little direct evidence concerning the actions of ATP on nociceptive afferents, and that which exists does not appear to support a role for $\alpha\beta$meATP-sensitive P2X receptors in nociception. For example, in the cat, polymodal nociceptors innervating the cornea (Dowd *et al*., 1997a), and sensory afferents innervating tooth pulp (Matthews *et al*., 1997), were not excited by $\alpha\beta$meATP. There is currently no direct evidence linking functional P2X receptors and peripheral nociceptive neurones in rat.

Levels of ATP are increased in inflamed or damaged tissues (Gordon, 1986) and the nucleotide is found in the synovial fluid of patients with arthritis (Ryan *et al*., 1991; Park *et al*., 1996). The actions of ATP on sensory neurons *in vitro* are enhanced by acidification (Li *et al*., 1996, 1997), and a fall in synovial pH occurs in human rheumatoid arthritis (Farr *et al*., 1985) and in experimentally-induced inflammatory arthritis (Tulamo *et al*., 1989). Thus, locally released ATP may contribute to pain by acting on P2X receptors associated with nociceptive afferents in the joint, and this action could be enhanced in inflammatory conditions such as arthritis. Alternatively, desensitization or down-regulation of P2X receptors might result from the continued presence of ATP within the inflamed joint capsule. What effect exogenous ATP actually has on nociceptive afferents innervating chronically inflamed joints needs to be established.

The aim of our study was to test the hypothesis that functional P2X receptors are expressed on primary afferent articular nociceptors. We recorded the neural discharge of nociceptors innervating the rat knee joint and measured the response to locally injected P2 receptor agonists. We also determined whether thes0.e responses were modified in joints with adjuvant-induced arthritis, a commonly used model for human arthritis (Rainsford, 1982). A preliminary account of some of the work has been presented (Dowd *et al*., 1997b).

[3] Author for correspondence.

Methods

Experiments, licensed under U.K. Home Office regulations, were performed on 30 normal and 18 arthritic male Wistar rats (body weight range 240–380 g; mean ± s.e.mean 325 ± 11 g).

Induction of arthritis

Freund's Complete Adjuvant (FCA, 0.10–0.15 ml of 1 mg ml^{-1} *Mycobacterium tuberculosis* in paraffin oil, Sigma) was injected intra-articularly into the left knee joint of rats under halothane anaesthesia (5% in oxygen). Animals were used for electrophysiological recordings 14–21 days post-induction at which time a mild persistent unilateral arthritis was present and associated with swelling of the knee (mean increase in diameter of 30% from 1.0 ± 0.01 cm to 1.3 ± 0.03 cm, $n = 18$).

Surgical procedures

Animals were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p., supplemented hourly with 6 mg i.v. *via* a cannula inserted into the right femoral vein). The trachea and right carotid artery were cannulated, and respiration and arterial blood pressure were continuously monitored. Body temperature was maintained at 38°C by an automated heating blanket connected to a thermistor probe inserted into the rectum. A cannula was inserted into the lower abdominal artery through the right femoral artery for close arterial injection of drugs to the left knee joint.

Electrophysiological recordings

The left leg was fixed to a support and the skin on the medial aspect of the limb was cut to expose three branches of the medial articular nerve (MAN) where they leave the saphenous nerve. The skin flaps were used to form a trough, which was filled with heavy paraffin oil.

The saphenous nerve was cut centrally to eliminate efferent neural activity. One of the branches of the MAN was dissected from the underlying connective tissue and neural discharge from small filaments containing 1–4 afferent fibres was recorded using bipolar platinum-iridium wire electrodes as described previously for the ankle joint (Grubb *et al*., 1991). Briefly, neural activity was recorded digitally on videotape and analysed off-line using a pulse-height voltage discriminator linked to a personal computer operating Spike 2 (CED, Cambridge) software. Single action potentials, identified by the size and shape of the spike, were counted separately. The receptive fields of the afferents were identified by probing the knee joint's capsule with a hand held plastic probe to activate fibres with mid-high mechanical thresholds. However, because ATP is released from damaged tissues, the mechanical thresholds were not systematically measured to avoid damaging or desensitizing the capsular tissue. Conduction velocities (conduction distance/action potential delay) were determined at the end of an experiment from the time taken for the action potential to reach the recording electrodes following a depolarizing stimulus applied to the receptive field using a stimulating electrode.

Drug administration

For intra-arterial (i.a.) injections, agonists were injected in a volume of 0.1 ml, washed in with 0.2 ml of saline (0.9% w/v sodium chloride), the injection being completed within 2 s. Repeatable responses to agonists were obtained before using antagonists. The minimum interval between successive agonist doses was 20 min. Antagonist was injected i.a. over 10 s (0.1 ml 100 g^{-1}-body weight) at least 10 min before agonists were re-tested. In some cases it was necessary to administer an additional dose of antagonist if its effects were observed to be waning.

In six normal animals, drugs were administered by intra-articular (i.art.) injection to the knee joint using a 26-gauge needle inserted through the infrapatellar ligament just beneath the patella. To determine whether the presence of the needle in the knee joint influenced neural activity, spontaneous afferent discharge and that evoked by agonists was recorded prior to insertion of the needle. The needle, fitted to a syringe containing drug solution, was then inserted into the joint space and secured with a clamp. Drawing 20–30 μl of air into the syringe after the drug solution prevented leakage of drug into the joint during the subsequent recording. Spontaneous activity and responses to agonists were then recorded. Because the rat knee joint volume is 0.15–0.20 ml, we performed only one or two injections (0.1 ml i.art.) per knee.

Data analysis

The effect of a drug or vehicle injection was determined by comparing the action potential discharge frequency following drug injection with that in the 15 s pre-injection period. Data are expressed as the mean change in action potential frequency ± s.e.mean. Marked receptor desensitization and cardiovascular effects can occur following injection of $\alpha\beta$meATP (McQueen *et al*., 1997), so high doses (> 200 nmol) were not routinely used. Consequently, it was difficult to establish the true maximum response to this agonist, and data from individual experiments were expressed as *apparent* ED_{50} values. Differences between means were analysed statistically using the Mann-Whitney test and the null hypothesis rejected at the $P \geqslant 0.05$ level.

Drugs

Adenosine 5′ triphosphate disodium salt (ATP), $\alpha\beta$-methylene ATP lithium salt and 8-methyl-N-vanillyl-6-nonenamide (capsaicin) were purchased from Sigma and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid tetrasodium (PPADS) was from Tocris. All drugs were dissolved in phosphate buffered saline (PBS) except for capsaicin, which was dissolved in Tween 80 (10% v/v), ethanol (10% v/v) and PBS.

Results

Characterization of afferent fibres

Neural discharge was recorded from a total of 104 single afferent fibres from 42 knee joints. The afferents were classified as C-fibre polymodal nociceptors (61%) or Aδ-mechanonociceptors (39%) based on their conduction velocities, mechanosensitivity and whether or not they were activated by the algogen, capsaicin. C-fibre polymodal nociceptors were slowly conducting (1.08 ± 0.17 ms^{-1}), excited by capsaicin (10 nmol: 24.0 ± 3.9 impulses s^{-1}), and were activated by mechanical stimulation of the joint capsule. Aδ-mechanonociceptors were faster conducting (3.42 ± 0.54 ms^{-1}) and did not respond to capsaicin. Some Aβ-mechanoreceptors were also identified (low threshold mechanosensitive units with conduc-

tion velocities greater than 10 ms^{-1}), but these were not studied.

Comparison of basal discharge in nociceptive afferents from normal and arthritic joints

We recorded from 57 afferent fibres (57% C, 43% Aδ) innervating normal knee joints and 47 (53% C, 47% Aδ) innervating arthritic knee joints. Previous electrophysiological studies in the rat ankle joint have shown two characteristic features in adjuvant-induced arthritis. These are: (1) that nerves innervating arthritic joints have a higher proportion of spontaneously active afferents than those innervating normal joints (2) the rate of discharge of those afferents which are spontaneously active is higher in arthritic joints (Guilbaud *et al*., 1985). In our experiments Aδ-mechanonociceptors innervating normal knee joints ($n=23$) were always silent, and in arthritic joints only 1 of 17 (6%) Aδ afferents was spontaneously active (0.1 impulses s^{-1}). In contrast, 16% (5/34 afferents) of C-fibre polymodal nociceptors innervating normal knee joints had low basal levels of spontaneous activity (0.10 ± 0.01 impulses s^{-1}), whereas 32% (10/31 afferents) of those innervating arthritic joints were active and discharged at significantly higher rate (0.39 ± 0.01 impulses s^{-1}, $P<0.05$ versus normal joints).

Histological assessment of the injected and contralateral knee joints of six rats (one normal, one vehicle-treated, and four injected with adjuvant) confirmed the presence of an inflammatory lesion manifest as an inflammatory cell infiltrate, synovial proliferation, fibroplastia, oedema and new bone formation in the adjuvant-injected knees (data not shown).

Responses to αβmeATP in normal and arthritic knee joints

The P2X receptor agonist, $\alpha\beta$meATP (1-600 nmol i.a.), evoked an increase in action potential discharge frequency in 55% of C-fibre polymodal nociceptors and 65% of Aδ-mechanonociceptors recorded from normal joints. Pooled data are shown because the responses evoked from both types of afferent fibre were similar. The increase in discharge following $\alpha\beta$meATP was rapid in onset, of short duration (Figure 1) and was dose-related (Figure 2); injection of vehicle evoked no response (PBS 0.1 ml: 0.01 ± 0.01 impulses s^{-1}). Injection of higher doses of $\alpha\beta$meATP, or repeating lower doses at short (<15 min) intervals, caused tachyphylaxis, i.e. a loss of responsiveness to previously effective doses (but not to mechanically-induced excitation) which lasted for about 20–30 min.

$\alpha\beta$meATP also evoked an increase in action potential discharge in 46% of C-fibres and 89% of Aδ-fibres recorded from arthritic joints. Adjuvant-induced arthritis did not affect the mean apparent ED_{50} for excitation of the afferents caused by $\alpha\beta$meATP (normal: 31 ± 12 nmol, $n=9$; arthritic: 57 ± 24 nmol, $n=5$). Similarly, as shown in Figure 3, the magnitude, latency to onset, and duration of the response evoked by a single mid-range dose of $\alpha\beta$ATP (60 nmol i.a.) did not differ significantly between normal and arthritic joints.

Responses to ATP in normal and arthritic knee joints

ATP, the endogenous ligand for P2X receptors, also evoked a rapid short-lasting increase in action potential discharge in 51% of C-fibre polymodal nociceptors and 81% of Aδ-mechanonociceptors on which it was tested in normal joints, and in 43% of C-fibres and 88% of Aδ-fibres in arthritic joints. Adjuvant-induced arthritis of the knee joint did not affect the magnitude, latency to onset, or duration of the response evoked by a single high dose of ATP (2000 nmol; see Figure 3). During experiments in which ATP and $\alpha\beta$meATP were both tested ($n=58$ afferents), fibres which were excited by ATP also invariably responded to $\alpha\beta$meATP and there was no significant difference between the responses evoked by the two nucleotides with respect to response amplitude, onset latency and duration (Figure 3). Seventeen of the fibres that responded to both $\alpha\beta$meATP and ATP were C-fibre polymodal nociceptors that also responded to capsaicin. The latency to onset of responses to $\alpha\beta$meATP and ATP did not differ significantly from that to capsaicin in these fibres ($\alpha\beta$meATP 60 nmol: 0.8 ± 0.1 s; ATP 2000 nmol: 0.6 ± 0.3 s; capsaicin 10 nmol: 1.1 ± 0.2 s). ATP also evoked a delayed increase in discharge which may be due to activation of adenosine receptors following metabolism of ATP by ectonucleotidase to adenosine (data not shown).

Effect of αβmeATP and ATP injected i.art. into the knee joint

To confirm that we were investigating responses in joint afferents, we injected $\alpha\beta$meATP and ATP directly into the knee. Injection of either substance evoked a transient increase in action potential discharge, whereas i.art. injection of the same volume of vehicle had no effect ($\alpha\beta$meATP 60 nmol: 4.4 ± 1.0 impulses s^{-1}; ATP 2000 nmol: 9.9 ± 2 impulses s^{-1};

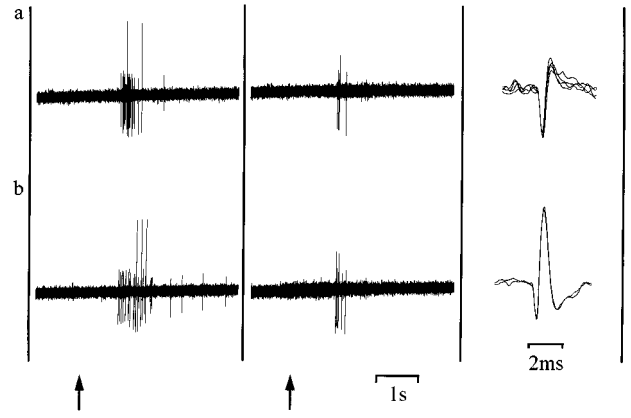

Figure 1 Oscilloscope traces showing the discharge evoked from a nerve filament innervating a normal knee joint by close-arterial injection (at arrow) of (a) $\alpha\beta$meATP (60 nmol) and (b) ATP (2000 nmol). The first panel shows the control responses and the second panel shows the responses 10–30 min after injection of the P2 purinoceptor antagonist, PPADS (16 μmol kg^{-1} i.a.). The third panel shows the two action potentials that were counted.

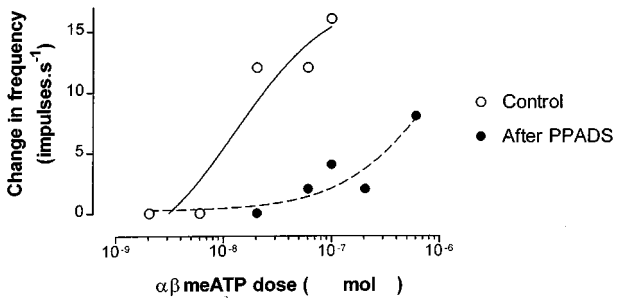

Figure 2 Dose-related increase in afferent discharge evoked by close-arterial injection of $\alpha\beta$meATP in a single C-fibre innervating a normal knee joint before and after PPADS (16 μmol kg^{-1} i.a.).

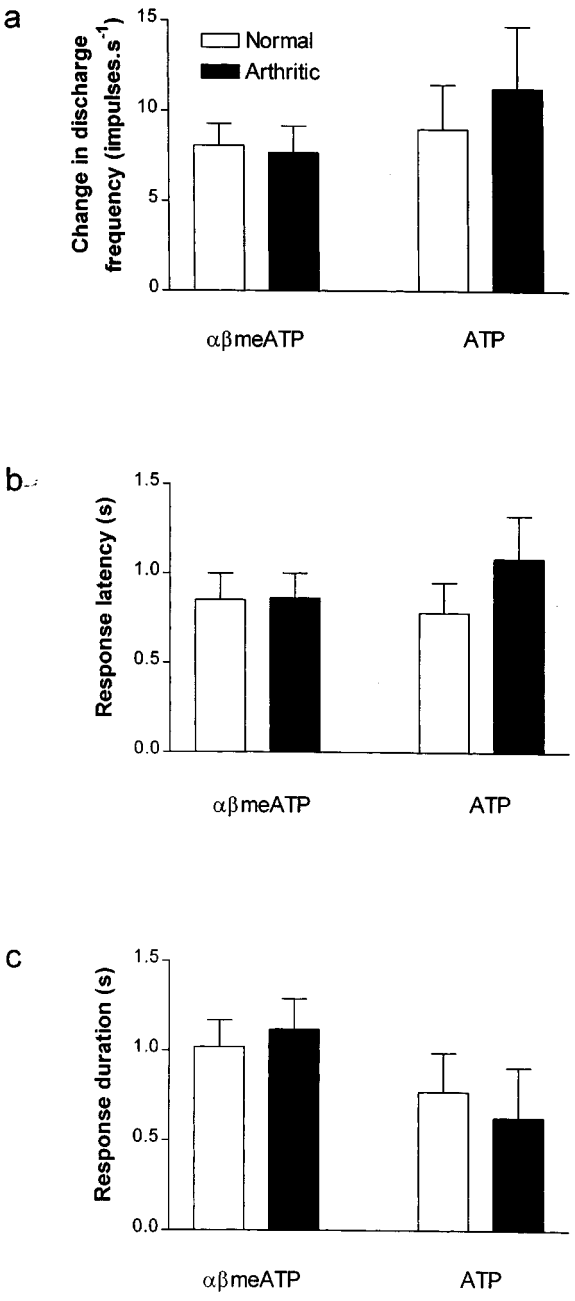

Figure 3 Summary of (a) increase in nociceptive discharge (b) latency to onset of the excitation and (c) duration of the response following i.a. injection of either $\alpha\beta$meATP (60 nmol) or ATP (2000 nmol). Data is shown for normal ($\alpha\beta$meATP: $n=14$; ATP $n=7$) or chronically arthritic joints ($\alpha\beta$meATP: $n=15$; ATP $n=7$). There was no significant difference with either agonist between the responses obtained from normal and arthritic joints.

saline (PBS) 0.1 ml: 0.01 ± 0.02 impulses s^{-1}; $n=3$ for each). The presence of the needle in the knee joint for the duration of the recording had no significant effect on the spontaneous discharge of the afferents (control: 0.01 ± 0.02 impulses s^{-1}; after insertion of needle: 0.01 ± 0.03 impulses s^{-1}, $n=9$ units).

Effects of PPADS on the nociceptive responses to αβmeATP and ATP

The increase in discharge evoked by $\alpha\beta$meATP (1-600 nmol i.a.) in normal and arthritic joints was antagonized by the P2X receptor antagonist, PPADS (16 μmol kg^{-1}, i.a.) as illustrated

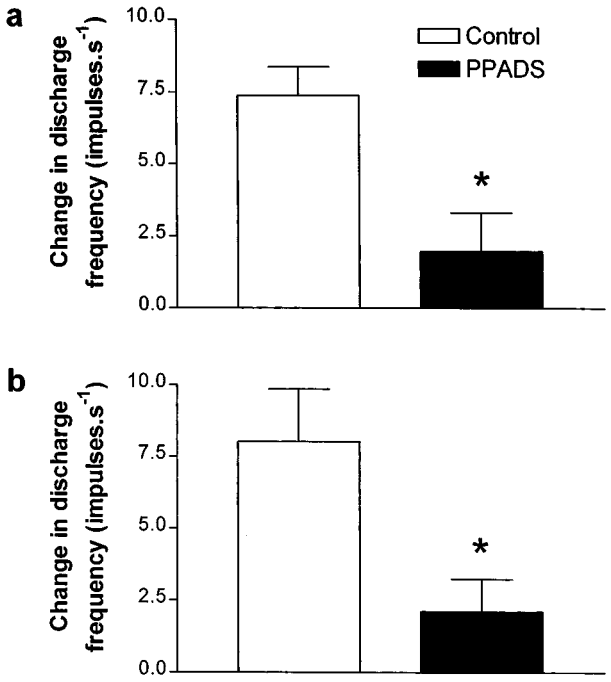

Figure 4 Response evoked by close-arterial injection of (a) $\alpha\beta$meATP (60 nmol) and (b) ATP (2000 nmol) before and after PPADS (16 μmol kg^{-1} i.a., 10 min pre-treatment). Pooled data from afferents innervating normal and arthritic knee joints ($\alpha\beta$meATP: $n=27$ afferents before, $n=11$ afferents after; ATP: $n=10$ afferents before, $n=6$ afferents after). Columns represent mean$\pm$s.e.mean. $^*P<0.05$ compared to control responses, Mann-Whitney test.

in Figure 1, Figure 2 and Figure 4. The mean apparent ED_{50} for $\alpha\beta$meATP was 31 ± 12 nmol ($n=9$) before and 258 ± 84 nmol ($n=4$) after PPADS ($P<0.01$). PPADS also antagonized the initial response to ATP (Figure 4), without affecting the delayed ATP response (data not shown).

Effect of PPADS on spontaneous discharge from nociceptive afferents innervating arthritic joints

In order to establish whether endogenous ATP contributed to the increased basal discharge, the effect of PPADS on spontaneous discharge was examined in nine of the eighteen arthritic joints. Activity was recorded from 17 afferent fibres; nine of which were C-fibre polymodal nociceptors, five of which were excited by ATP. The other eight were Aδ-mechanonociceptors which lacked any spontaneous discharge, and six of these were responsive to ATP. Four (44%) of the C-fibres were spontaneously active, and two were excited by ATP. Administration of PPADS (16 μmol kg^{-1}, i.a.) did not reduce the firing rate of the four C-fibre afferents (frequency before PPADS: 0.5 ± 0.5 impulses s^{-1}; 10 min after PPADS: 0.4 ± 0.4 impulses s^{-1}). However, PPADS would only be expected to influence the two spontaneously active ATP-positive fibres: the antagonist reduced the discharge in one of the recordings, but it increased it slightly in the other (unit 1 before: 0.50 impulses s^{-1}; after 0.3 impulses s^{-1}, unit 2: before 0.3 impulses s^{-1}; after 0.4 impulses s^{-1}).

Discussion

The main finding from this *in vivo* pharmacological study is that ATP and $\alpha\beta$meATP both cause a rapid short-lasting excitation of a sub-population of nociceptive afferent nerve

fibres innervating the rat knee joint. Chronic adjuvant-induced arthritis did not significantly affect the responsiveness of these joint sensors to the purinoceptor agonists studied. This evidence supports the hypothesis we were testing, namely that functional P2X receptors are present on the peripheral terminals of primary afferent articular nociceptors. Activation of these purinoceptors excites nociceptive afferents in normal as well as in chronically inflamed arthritic joints.

The delay to onset of joint afferent responses to intra-arterially injected alogogens gives some indication of the transduction mechanism through which these substances mediate their effects. Thus, excitation with fast onset is typically observed with algogens known to mediate their effects through specific ion channels receptors expressed on the sensory nerve terminals, whereas slow onset responses are observed with algogens acting through G-protein coupled receptors expressed on the nerve terminal (Birrell *et al*., 1990). We found that capsaicin rapidly increased afferent discharge with a delay to onset which was similar to that observed following injections of $\alpha\beta$meATP or ATP in the same recordings. Since capsaicin acts directly on the sensory nerve terminal via a specific vanilloid ion-channel receptor (Caterina *et al*., 1997), it is probable that the fast responses to $\alpha\beta$meATP and ATP are also mediated via direct actions on the afferent nerve terminals, rather than through an intermediary. We also established that injection of $\alpha\beta$meATP or ATP directly into the knee joint evoked rapid excitation, which provides additional evidence that responses observed following i.a. injection of the agonists were not secondary to effects on the vasculature.

In the dorsal root ganglia, where the cell bodies for knee joint nociceptors are located, mRNA for six of the ATP-gated cation channel receptors ($P2X_{1\text{-}6}$) are expressed (Collo *et al*., 1996). Only two of these receptors, namely the $P2X_1$ and $P2X_3$ subtypes, are sensitive to the P2X agonist, $\alpha\beta$meATP, and the non-selective P2 antagonist, PPADS. There are currently no selective pharmacological tools available for use *in vivo* that would enable us to discriminate between responses mediated by these subtypes. However, previous studies have shown $P2X_3$ mRNA to be selectively expressed in sensory ganglia (Lewis *et al*., 1995) and nociceptive afferents in the rat tooth pulp have recently been shown to possess immunoreactivity for the $P2X_3$, but not the $P2X_1$, receptor subtype (Cook *et al*., 1997). This restricted localization of the $P2X_3$ receptor suggests that it may play an important role in the initiation of primary afferent depolarization.

However, the properties of homomeric $P2X_3$ receptors expressed in HEK293 cells are very different from those of the native receptor in isolated sensory (nodose ganglion) neurones (see Lewis *et al*., 1995), and this had led to the suggestion that heteropolymerization of P2X receptors may occur *in vivo*. The only heteropolymer which displays the phenotype of the native P2X receptor of sensory neurones ($\alpha\beta$meATP sensitive, slowly desensitizing and increases in affinity for ATP with decreasing pH) is the $P2X_{2/3}$ heteropolymer (Lewis *et al*., 1995; Stoop *et al*., 1997). In our experiments, the excitation of joint nociceptors by $\alpha\beta$meATP and ATP lasted for approximately 0.5–1.0 s which is longer than expected if the responses were mediated by homomeric $P2X_3$ since responses to ATP at these receptors desensitize within milliseconds. Thus it is possible that the $P2X_{2/3}$ heteropolymer mediated the fast excitation evoked by $\alpha\beta$meATP and ATP in our experiments.

One of the features of the ATP-evoked response in cultured sensory neurones is an augmentation of the response with decreasing pH (Li *et al*., 1996, 1997). This may mean that the fall in tissue pH during inflammation (see Introduction) could sensitise nociceptive terminals to ATP. However, we found the overall response of joint nociceptors to injected $\alpha\beta$meATP and ATP was not significantly affected by the presence of adjuvant arthritis in the knee joint. Although the pH of synovial fluid was not measured in our experiments, we consider it reasonable to assume that it fell because pH is known to decrease in rheumatoid arthritis (Farr 1985) and during synovitis associated with infectious arthritis in horses (Tulamo *et al*., 1989). Our results suggest that the augmentation of ATP-evoked responses by the decrease in pH observed in sensory neurones *in vitro* does not occur during adjuvant-induced arthritis *in vivo*. Alternatively, pH-sensitive P2X receptors are not responsible for the excitation mediated by ATP in our experiments.

Inflammation is also associated with an increase in the level of extracellular ATP which can be released from damaged cells (Gordon, 1986), platelets (Born & Kratzer, 1984), some inflammatory cells (Di Virgilio *et al*., 1996; Ferrari *et al*., 1997) and perhaps the sensory nerve terminal itself (Holton, 1959). The continuous exposure of nociceptors to endogenous ATP could cause chronic desensitization, equivalent to that observed in some inflammatory cells where the P2X-mediated response is only unmasked following pre-treatment with the ATP-metabolising enzyme apyrase which metabolizes the ATP released from the cells themselves (see Di Virgilio *et al*., 1996). However, in the chronically inflamed arthritic knee joints, the nociceptive afferents remained sensitive to the P2X receptor agonists, suggesting that either high levels of extracellular ATP are not found in the inflamed joint in this model of arthritis, or that endogenous ATP does not desensitize the afferent P2X receptor involved. Since nociceptive afferents retain their sensitivity to ATP during chronic inflammation, endogenously released ATP could contribute to the associated pain and hyperalgesia by exciting them.

The pain and hyperalgesia of adjuvant-induced arthritis is manifest as an increase in the number and rate of discharge of spontaneously active nociceptive afferents (Guilbaud *et al*., 1985; McQueen *et al*., 1991). In our experiments, basal discharge was higher in afferents innervating arthritic joints, but it was not possible to establish the extent to which endogenous ATP contributed to this sensitization because only a small proportion of the spontaneously active afferents were ATP-sensitive. Further studies, perhaps involving a more severe arthritis to cause greater ATP release from more severely inflamed tissues, are required. In addition, interpretation of experiments involving PPADS is complicated by the fact that, in common with other P2 receptor antagonists, it also inhibits the enzymes responsible for ATP metabolism (Khakh *et al*., 1995). Simultaneous antagonism of P2X receptors and inhibition of ATP breakdown leads to apparent antagonist insensitivity in some preparations (Crack *et al*., 1994). The development of selective P2X receptor antagonists, which lack this ectonucleotidase-blocking property, would better answer the question of whether endogenous ATP contributes to the sensitization of nociceptors seen in inflammatory models.

It is worth speculating as to why activation of P2X receptors excites nociceptors in joints, whereas this did not occur when $\alpha\beta$meATP was tested electrophysiologically on cat corneal nociceptors (Dowd *et al*., 1997a) or cat tooth pulp afferents (Matthews, 1997). Species differences could be invoked to explain the discrepancies, or fundamental differences may exist in the populations of P2X receptor subtypes associated with particular afferent nerve terminals at different sites in the body. Further studies would be needed to explore these possibilities. However, since the response elicited is dependent on the concentration of drug, the speed with which the agonists reach the receptor is important; slow penetration

may be associated with receptor desensitization, which is one possible explanation for the differences observed. We may have detected P2X-mediated excitation of joint afferents by our use of close-arterial bolus injections to deliver optimal concentrations of agonists to the receptors.

Our results support the hypothesis that functional $\alpha\beta$meATP-sensitive P2X receptors are present on a subpopulation of peripheral nociceptive afferent nerves in the rat knee joint. ATP can excite these receptors in normal and in chronically inflamed joints. This data supports the suggestion that P2X receptors are involved in the initiation of nociception and pain and that they may represent a potential target for the development of new analgesics.

References

BIRRELL, G.J., MCQUEEN, D.S., IGGO, A., COLEMAN, R.A. & GRUBB, B.D. (1990). PGI2-induced activation and sensitisation of articular mechanonociceptors. *Neurosci Lett.,* **124,** 5–8.

BLAND-WARD, P.A. & HUMPHREY, P.P.A. (1997). Acute nociception mediated by hindpaw P2X receptor activation in the rat. *Br. J. Pharmacol.,* **122,** 365–371.

BLEEHAN, T. & KEELE, C.A. (1977). Observations on the algogenic actions of adenosine compounds on the human blister base preparation. *Pain,* **3,** 267–377.

BORN, G.V., KRATZER, M.A. (1984). Source and concentration of extracellular adenosine triphosphate during haemostasis in rats, rabbits and man. *J. Physiol.,* **354,** 419–429.

BURNSTOCK, G. & WOOD, J.N. (1996). Purinergic receptors: their role in nociception and primary afferent neurotransmission. *Curr. Opin. Neurobiol.,* **6,** 526–532.

CATERINA, M.J., SCHUMACHER, M.A., TOMINAGA, M., ROSEN, T.A., LEVINE, J.D. & JULIUS, D. (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature,* **389,** 816–824.

CHEN, C.-C., AKOPIAN, L.S., COLQUOHOUN, D., BURNSTOCK, G. & WOOD, J.N. (1995). A P2X purinoceptor expressed by a subset of sensory neurons. *Nature,* **377,** 428–431.

COLLO, G., NORTH, R.A., KAWASHIMA, E., MERLOPICH, E., NEIDHART, S., SUPRENANT, A. & BUELL, G. (1996). Cloning of $P2X_5$ and $P2X_6$ receptors and the distribution and properties of an extended family of ATP-gated ion channels. *J. Neurosci.,* **16,** 2495–2507.

COOK, S.P., VULCHANOVA, L., HARGREAVES, M.N., ELDE, R. & MCCLESKEY, E.W. (1997). Distinct ATP receptors on pain-sensing and stretch-sensing neurones. *Nature,* **387,** 505–508.

CRACK, B.E., BEUKERS, M.W., MCKECHNIE, K.C., IJZERMAN, A.P. & LEFF, P. (1994). Pharmacological analysis of ecto-ATPase inhibition: evidence for combined enzyme inhibition and receptor antagonism in P2X-purinoceptor ligands. *Br. J. Pharmacol.,* **113,** 1432–1438.

DI VIRGILIO, F., FERRARI, D., CHIOZZI, P., FALZONI, S., SANZ, J.M., DAL SUSINO, M., MUTINI, C., HANAU, S. & BARICORDI, O.R. (1996). Purinoceptor function in the immune system. *Drug Dev. Res.,* **39,** 319–329.

DOWD, E., GALLAR, J., MCQUEEN, D.S., CHESSELL, I.P., HUMPHREY, P.P.A. & BELMONTE, C. (1997a). Nociceptors of the cat and rat cornea are not excited by P2X purinoceptor agonists. *Br. J. Pharmacol.,* **122,** 348P.

DOWD, E., MCQUEEN, D.S., CHESSELL, I.P. & HUMPHREY, P.P.A. (1997b). Activation by P2X purinoceptor agonists of sensory nerves innervating the rat knee joint. *Br. J. Pharmacol.,* **122,** 286P.

FARR, M., GARVEY, K., BOLD, A.M., KENDALL, M.J. & BACON, P.A. (1985). Significance of the hydrogen ion concentration in synovial fluid in rheumatoid arthritis. *Clin. Exp. Rheumatol.,* **3,** 99–104.

FERRARI, D., CHIOZZI, P., FALZONI, S., HANAU, S. & DI VIRGILIO, F. (1997). Purinergic modulation of interleukin-1β release from microglial cells stimulated with bacterial endotoxin. *J. Exp. Med.,* **185,** 579–582.

FREDHOLM, B.B., ABBRACCHIO, M.P., BURNSTOCK, G., DALY, J.W., HARDEN, T.K., JACOBSON, K.A., LEFF, P. & WILLIAMS, M. (1994). Nomenclature and classification of purinoceptors. *Pharmacol. Rev.,* **46,** 143–156.

GORDON, J., (1986). Extracellular ATP: effects, sources and fate. *Biochem J.,* **233,** 309–319.

GRUBB, B.D., BIRRELL, G.J., MCQUEEN, D.S. & IGGO, A. (1991). The role of PGE_2 in the sensitisation of mechanonociceptors in normal and inflamed ankle joints of the rat. *Exp. Brain Res.,* **84,** 383–392.

GUILBAUD, G., IGGO, A. & TEGNER, R. (1985). Sensory receptors in ankle joint capsules of normal and arthritic rats. *Exp. Brain. Res.,* **58,** 29–40.

HOLTON, P. (1959). The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. *J. Physiol.,* **145,** 494–504.

JAHR, C.E. & JESSELL, T.M. (1983). ATP excites a subpopulation of rat dorsal horn neurones. *Nature,* **304,** 730–733.

KHAKH, B.S., MICHEL, A.D. & HUMPHREY, P.P.A. (1995). Inhibition by ectoATPase and Ca-ATPase in rat vas deferens by P2 purinoceptor antagonists. *Br. J. Pharmacol.,* **115,** 2P.

LEWIS, C., NEIDHART, S., HOLY, C., NORTH, R.A., BUELL, G. & SUPRENANT, A. (1995). Coexpression of P2X2 and P2X3 receptor subunits can account for ATP-gated currents in sensory neurons. *Nature,* **377,** 432–435.

LI, C., PEOPLES, R.W. & WEIGHT, F.F. (1996). Acid pH augments excitatory action of ATP on a dissociated mammalian sensory neuron. *NeuroReport.,* **7,** 2151–2154.

LI, C., PEOPLES, R.W. & WEIGHT, F.F. (1997). Enhancement of ATP-activated current by protons in dorsal root ganglion neurons. *Pflügers Arch.,* **433,** 446–454.

MATTHEWS, B., LI, F., KHAHK, B.S. & VONGSAVAN, N. (1997). Effects of ATP on sensory receptors in the dental pulp of cats. *J. Physiol.,* **504,** 128P.

MCQUEEN, D.S., BOND, S.M., MOORES, C., CHESSELL, I.P., HUMPHREY, P.P.A. & DOWD, E. (1998). Activation of P2X receptors for adenosine triphosphate evokes cardiorespiratory reflexes in anaesthetised rats. *J. Physiol.,* **507,** 843–855.

MCQUEEN, D.S., IGGO, A., BIRRELL, G.J. & GRUBB, B.D. (1991). Effects of paracetamol and aspirin on neural activity of joint mechanonociceptors in adjuvant arthritis. *Br. J. Pharmacol.,* **104,** 178–182.

MCQUEEN, D.S., MOORES, C., DOWD, E., BOND, S.M., CHESSELL, I.P. & HUMPHREY, P.P.A. (1997). P2X receptor activation evokes a Bezold-Jarisch like reflex in anaesthetised rats. *Br. J. Pharmacol.,* **122,** 246P.

NORTH, R.A. & BARNARD, E.A. (1997). Nucleotide receptors. *Curr. Op. Neurobiol.,* **7,** 346–357.

PARK, W., MASUDA, I., CARDENAL-ESCARCENA, A., PALMER, D.L. & MCCARTHY, D.J. (1996). Inorganic pyrophosphate generation from adenosine triphosphate by cell-free human synovial fluid. *J. Rheumatol.,* **23,** 665–671.

RAINSFORD, K.D. (1982). Adjuvant polyarthritis in rats: is this a satisfactory model for screening anti-arthritic drugs? *Agents & Actions,* **12,** 452–458.

RYAN, L.M., RACHOW, J.W. & MCCARTY, D.J. (1991). Synovial fluid ATP: a potential source for the production of inorganic pyrophosphate. *J. Rheumatol.,* **18,** 716–720.

STOOP, R., SURPRENANT, A. & NORTH, R.A. (1997). Different sensitivities to pH of ATP-induced currents at four cloned P2X receptors. *J. Neurophysiol.,* **78,** 1837–1840.

TREZISE, D.J., KENNEDY, I. & HUMPHREY, P.P.A. (1993). Characterization of purinoceptors mediating depolarisation of rat isolated vagus nerve. *Br. J. Pharmacol.,* **110,** 1055–1060.

TULAMO, R.M., BRAMLAGE, L.R. & GABEL, A.A. (1989). Sequential clinical and synovial fluid changes associated with acute infectious arthritis in the horse. *Equine Vet.* **21,** 325–331.

(Received March 24, 1998
Revised June 22, 1998
Accepted June 24, 1998)